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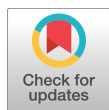
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## ANALYSIS

## Evolution of biological agents: how established drugs can become less safe

Changes to the manufacturing of biological agents can lead to drugs with different components from the original medicine tested in clinical trials, challenging assumptions about safety, say **David Hunt and colleagues**

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Biological drugs, such as recombinant proteins and monoclonal antibodies, have revolutionised the treatment of many diseases.<sup>1,2</sup> By definition, these agents are complex molecules produced in living cells, requiring multistep manufacturing processes such as cell culture, purification, stabilisation, and packaging.<sup>3</sup> Biological drugs (or “biologicals”) differ from small molecule drugs in that they are highly sensitive to changes in manufacturing. As a result, the safety profile of established biologicals can alter over time, posing a major challenge to the safety frameworks for such drugs.

### Biologicals change over time

Changes to the manufacturing of biologicals are surprisingly common, although healthcare professionals and patients are often unaware of them. For example, infliximab has undergone more than 35 alterations in its clinical lifetime of nearly 20 years.<sup>4</sup> Most of these changes will have been relatively minor, and the manufacturer will have demonstrated to the regulator that the biological was unchanged at a physicochemical level.<sup>3</sup> But not all changes are trivial, and some are introduced with the explicit intention of improving clinical parameters. Even if clinical trials are required after substantial alterations, they are typically short and rarely powered to detect adverse events that are uncommon or rare and serious.

“Biological evolution” describes how sequential manufacturing changes can cause the properties of a biological to diverge from the original (fig 1⇓).<sup>5</sup> For example, a recombinant protein might undergo sequential alterations to three key components—the active biological substance, the stabiliser, and the packaging—leading to a biological drug where all the main components have been changed. So does the drug retain the safety and efficacy profile of the original? Whether an object retains its fundamental properties after all its components have been sequentially replaced has puzzled both ancient and modern

philosophers (box 1). Today it poses an important practical question for drug safety.

This question challenges a dogma of drug safety: namely that older, established biologicals, which have been in widespread use for decades, have stronger evidence for their safety than their newer counterparts. This narrative is reinforced by the marketing materials of established brands, which promote a reassuring message of “decades of use” or “millions of patients treated.” The need for critical evaluation of this message has been heightened by the introduction of biosimilar agents, first in Europe and more recently in the US.<sup>2</sup> Biosimilars are new drugs that are similar to licensed biological agents, with no clinically meaningful differences in quality, efficacy, or safety.<sup>4</sup> The primary safety concern with biosimilars is that they are made by new manufacturing processes that have been tested in limited clinical trials. Their safety profiles are, therefore, perceived to be less well defined than their established counterparts.<sup>4</sup> But problems associated with limited testing of new manufacturing methods can equally affect older brands, despite reassuring marketing.

### Biological evolution can improve or worsen safety

Changes to biologicals are often driven by a manufacturer’s intention to improve an established biological drug, perhaps to be less immunogenic, better tolerated, or safer.<sup>6–9</sup> These improved parameters can be readily assessed in short trials, comprising a few hundred patients or fewer.<sup>3–10</sup> Such trials can provide reliable safety data about the short term clinical performance of evolved biological drugs but are not usually able to detect changes in long term safety or efficacy.<sup>11</sup> If the biological has diverged from the original in a beneficial

**Box 1: Trigger's broom and Theseus' ship**

"I've maintained this old broom for 20 years. It's had 17 new heads and 14 new handles in its time."—Trigger in *Only Fools and Horses*

"The ship wherein Theseus returned was preserved by the Athenians. For they took away the old planks as they decayed, putting in new and stronger timber in their place. This ship became a standing example among the philosophers . . . one side holding that the ship remained the same, and the other contending that it was not the same."

*The Life of Theseus*, Plutarch

parameter, it may also have diverged in a detrimental parameter—and this may be more difficult to detect.

Recombinant erythropoietin provides the first well documented example of how the safety profile of an evolved biological can deteriorate compared with the original.<sup>8</sup> This recombinant protein is widely used to treat anaemia and can cause pure red cell aplasia, a serious adverse event mediated by neutralising antibodies directed against erythropoietin.<sup>12 13</sup> In the first decade of postmarketing use, pure red cell aplasia was reported in only three patients worldwide who received recombinant erythropoietin. However, a sustained increase in incidence was subsequently observed in Europe, where an evolved preparation of erythropoietin- $\alpha$  had been introduced.<sup>8</sup> The preparation had undergone several sequential changes, in particular to the drug stabiliser and packaging, resulting in a change to protein immunogenicity. Although the exact cause was not definitively identified, concerted efforts to alter drug storage, injection, and manufacturing led to a drop in incidence of pure red cell aplasia.<sup>8</sup>

## A natural experiment of regulation

Regulatory authorities have differed in their approaches to the approval of evolved biologicals, setting the conditions for a potentially informative, if unintentional, natural experiment. Recombinant interferon beta has been used for the treatment of multiple sclerosis since 1998, and, although of only modest efficacy, it is considered to be well tolerated with a strong safety record.<sup>14–16</sup> As it entered its second decade of widespread use, the European Medicines Agency approved an evolved interferon beta preparation for use, whereas the US Food and Drug Administration did not.<sup>10</sup> The product had undergone two sequential changes—a new cell clone was introduced in 2003, and the stabiliser was changed to remove human serum components in 2007.<sup>10</sup> The new product had a better short term safety profile than the original, in particular a lower immunogenicity profile.<sup>6</sup> However, an unexpectedly high number of cases of renal failure caused by thrombotic microangiopathy were identified in Europe in association with the evolved product (fig 2).<sup>17 18</sup>

Thrombotic microangiopathy is a disease of small blood vessels that can cause fulminant organ failure. It is caused by a direct toxic effect of interferon beta protein and is exceptionally rare as a spontaneous disease.<sup>19 20</sup> It had not been identified as a problem with the original formulation of interferon beta, with no cases in extensive clinical trials covering many thousands of patient years and only three cases reported worldwide in almost a decade of postmarketing use.<sup>17</sup> By contrast, a strong, specific, and sustained signal of disproportionate reporting for thrombotic microangiopathy was seen in countries where the evolved preparation had been introduced (fig 2).<sup>17–21</sup>

## Flying under the radar?

Subsequent efforts to quantify this safety signal in populations in which efforts have been made to actively identify cases indicate that thrombotic microangiopathy can occur at an incidence of ~1 in 1000 patient years in patients with multiple sclerosis treated with evolved interferon beta and is associated

with a 10–20% mortality rate.<sup>17–22</sup> When serious adverse events of a similar magnitude have been detected early in pivotal clinical trials for other drugs for multiple sclerosis, manufacturers and regulators have responded quickly and vigorously, sometimes with temporary market suspension, to enable development of a robust risk mitigation programme.<sup>23</sup> By contrast, warnings about thrombotic microangiopathy associated with interferon beta have been incremental and of lower profile, with little in the way of active risk management.<sup>17 20</sup> This raises the question of whether serious adverse events associated with an evolved biological might trigger a less robust safety signal than the original.

Uncommon but serious adverse events are likely to be detected in the extensive clinical trials of an original biological product, which can span many thousand patient years. However, the much shorter trials of evolved products, with only a few hundred patient years of exposure, may not detect a single case.<sup>8–11</sup> This difference is crucial because even a single case in clinical trials can trigger further dedicated longitudinal study of the adverse event to accurately quantify risk.<sup>24</sup> If this opportunity is missed, then detecting and quantifying safety signals relies on spontaneous reporting, where data from the evolved product are potentially subject to a "double dilution" effect.<sup>25</sup> Firstly, only a small fraction of the true number of cases are reported because adverse event data are no longer actively collected.<sup>25</sup> Then the safety data from the evolved product may be further diluted by being pooled with extensive datasets from the original formulation. This is particularly concerning because adverse events associated with evolved biologicals may emerge at a time when safety data from the evolved product are dwarfed by that of the original (fig 3).

## Optimising pharmacovigilance

### Unbiased and proactive reporting

Spontaneous reporting is crucial for the generation of safety signals, so all doctors who prescribe and monitor biologicals should be aware of the phenomenon of biological evolution and guard against the potentially false reassurance of "a well established safety profile." Doctors should not assume that unexpected and serious risks cannot occur just because a familiar branded biological has been used for decades. Indeed, the longer the established safety profile, the stronger the potential diluting effect of pre-existing data (fig 3).

Direct communication between reporting clinicians, national drug safety agencies, and scientists can have an important role in the accurate definition of emerging drug induced syndromes. Thrombotic microangiopathy associated with interferon beta was recognised and reported differently between specialties; nephrologists recognised and reported it as "haemolytic uraemic syndrome," cardiologists as "malignant hypertension," and haematologists as "thrombotic thrombocytopenic purpura." These observations, informed directly from the bedside, facilitated more specific analyses of national and global safety datasets, enabling emergent safety signals to be more clearly defined than when broader case descriptions were used.<sup>17 22</sup>

Vigilance for adverse event reporting should be maintained throughout the lifetime of a drug. Healthcare professionals and patients may need to be more aware of major manufacturing changes, although alert systems can introduce their own reporting biases.<sup>26</sup> The pharmacovigilance model of spontaneous reporting may be fundamentally limited in its sensitivity to detect dynamic changes associated with biological evolution, highlighting the need for additional active surveillance systems.

## Biological registries

Several medical specialties recognised early the particular challenges of safety of biologicals, starting a culture of active surveillance led by healthcare professionals, exemplified by biological registries.<sup>27</sup> Although the pharmaco-epidemiology of biological agents is becoming ever more complex, a simple principle to evaluate risk of adverse events is to ensure accurate measures of case ascertainment and patient exposure. Biological registries achieve this through unbiased active reporting of adverse events, coupled to tracking of manufacturing changes. The British Society for Rheumatology established its biological registry over a decade ago. It has enabled more precise mapping of adverse events with biological agents, in particular complications with long term exposure.<sup>28</sup> UK neurologists opted instead to monitor the cost effectiveness of new biologicals for treating multiple sclerosis through a risk sharing scheme.<sup>15 29</sup> Patients with multiple sclerosis who developed serious complications from thrombotic microangiopathy associated with interferon beta were being actively monitored through this prospective cohort study, but efficacy rather than safety data were collected. Given the continued rise in use and complexity of biologicals, individual medical specialties should consider the adequacy of their current frameworks.

## Dedicated research

Biological evolution itself is understudied. Manufacturers and regulators readily accept that biological evolution can improve the short term safety profile of a drug yet are more reluctant to accept that it might alter the long term safety profile.<sup>22 30</sup> Some regulators and manufacturers have questioned the validity of strong safety signals with evolved biologicals because, by necessity, they rely on spontaneous reporting data rather than rigorous pharmacoepidemiological studies.<sup>22 30</sup> The absence of such studies is arguably the problem, hampering our understanding of the true extent of the challenges posed by evolved biologicals.

Notably, the examples we describe here led to meaningful alterations in the immunogenicity of a recombinant protein. On one hand, increased immunogenicity can cause serious adverse events, such as pure red cell aplasia.<sup>12 13</sup> On the other hand, reductions in immunogenicity can increase drug bioavailability and unmask toxic drug effects.<sup>20 31</sup>

Other key questions need to be answered: Which manufacturing changes are most likely to alter safety profiles? Are changes caused by a single, critical change, or the accumulation of many? Does this phenomenon particularly affect recombinant proteins or are monoclonal antibodies and vaccines also affected? The answers are critical to the design of an optimal monitoring framework, which balances cost, innovation, and safety.<sup>2</sup>

## Conclusion

Biological evolution has the potential to improve or worsen the safety profile of established biological medicines, but current safety frameworks are more likely to emphasise short term

improvements. More research is needed to understand the potential long term safety implications of such changes and to see if current safety frameworks might be further improved. In the meantime, all healthcare professionals should be aware of the phenomenon of biological evolution while executing their duty of drug safety reporting and should avoid complacency when prescribing established biological agents.

**Contributors and sources:** The authors are doctors who contributed to the identification of adverse drug events through spontaneous reporting, pharmacoepidemiological analyses, and basic science. NC identified the biological basis of the association between evolved erythropoietin and pure red cell aplasia.<sup>8-13</sup> DH and OF identified the biological basis of thrombotic microangiopathy associated with interferon beta and identified the association with evolved interferon.<sup>17</sup> DH and OF were awarded the Sir Derrick Dunlop prize for drug safety by the Medicines and Healthcare Products Regulatory Agency for identification of the association between interferon and thrombotic microangiopathy through spontaneous reporting. DH drafted the original draft of the manuscript and is the guarantor of the article.

**Competing interests:** We have read and understood BMJ policy on declaration of interests. DH is funded by the Wellcome Trust and is a member of the Medicines and Healthcare Products Regulatory Agency's neurology expert advisory group. OF is a principal investigator for studies by Chemocentryx and GSK. NC has been a consultant/speaker for Shire, Novartis, Sandoz, and BMS.

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### Key messages

- Biological drugs have complex manufacturing processes that can “evolve” over time
- Biological evolution can improve or worsen the safety profile of an established drug
- Current safety frameworks may not optimally detect uncommon but serious adverse effects of evolved biologicals
- Vigilance for adverse event reporting should be maintained throughout the lifetime of a biological drug

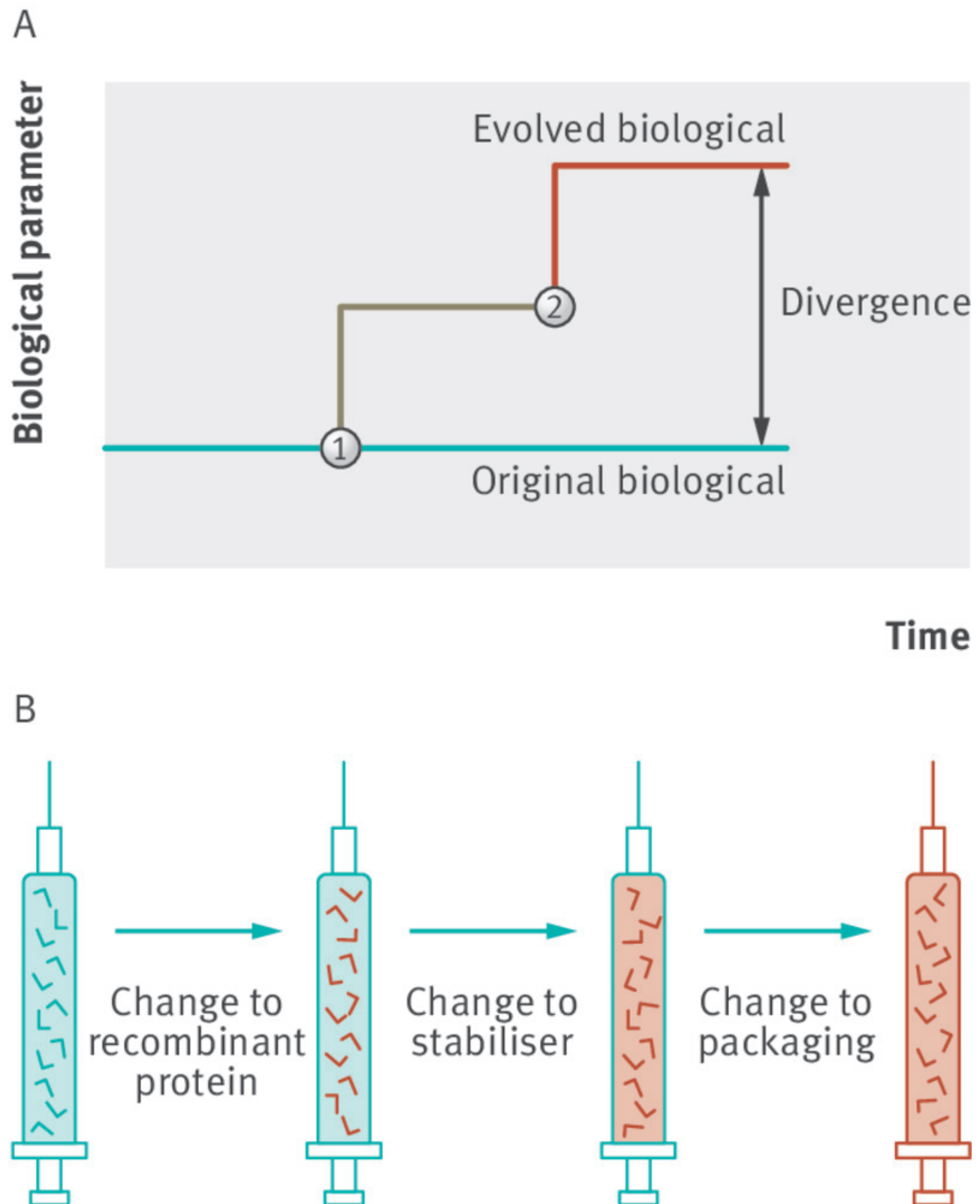
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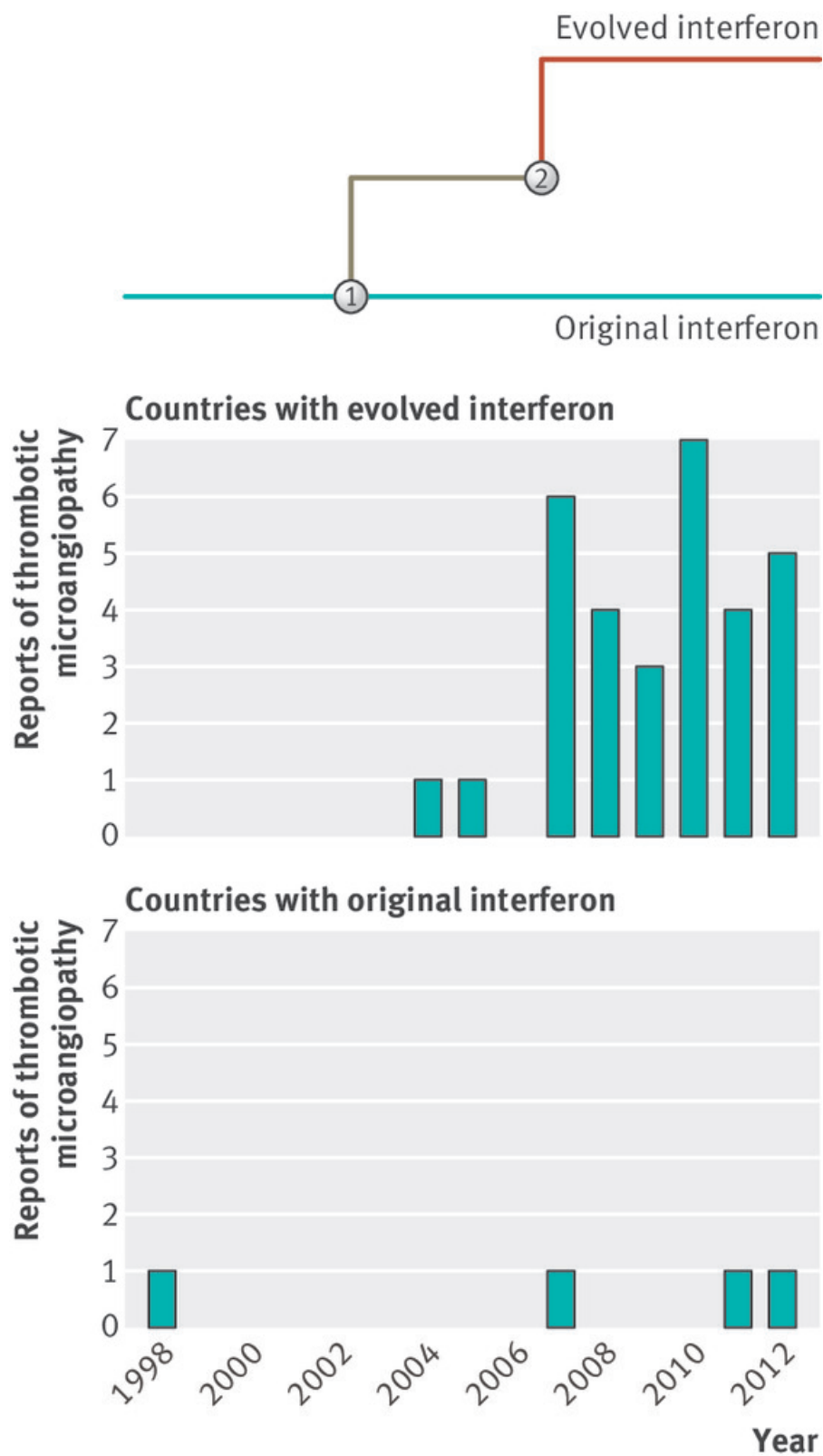
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## Figures



**Fig 1** Evolution of biological drugs. A) Definition. B) An example.



**Fig 2** Evolution of interferon beta and reports of thrombotic microangiopathy. Reprinted with permission.<sup>17</sup>



**Fig 3** The potential for dilution of safety signals from evolved biologicals. Exposure of an evolved biological can be dwarfed by the patient exposure of the original product. One person represents 500 patient years.